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TOXICITY AND BIOACCUMULATION OF CADMIUM AND LEAD IN AQUATIC INVERTEBRATES

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ABSTRACT

Cadmium toxicity and lead toxicity to four species of insects (Pteronarcys dorsata, Hydropsyche betteni, Brachycentrus *sp. and* Ephemerella *sp.*), *one snail* (Physa integra) *and one amphipod* (Gammarus pseudolimnaeus) *were determined during* 28-*day exposures. The* 28-*day* LC50 *values for cadmium-exposed snails and lead-exposed amphipods were eleven and four times lower than the* 7- *and* 4-*day* (96 *h*) *values for these metals, respectively. Lowest effect concentrations obtained after* 28 *days for cadmium-exposed mayflies* (Ephemerella *sp.*) *and snails and lead-exposed amphipods were similar to those affecting fish exposed over their complete life cycle in water of similar quality. Lethal threshold concentrations were not observed for species exposed to either metal, indicating that possible effects could occur at lower concentrations during longer exposure periods. Cadmium and lead concentrations in the animals tested generally increased with increasing water concentrations and were up to* 30,000 *and* 9000 *times greater than corresponding metal concentrations in the water.*

INTRODUCTION

Heavy metal contamination and its effects on freshwater aquatic life are well documented (National Academy of Sciences and National Academy of Engineering, 1973). Recent reviews by McKim *et al.* (1973, 1974, 1975, 1976) report an extensive amount of work on the effects of various pollutants, including heavy metals, on fish. However, information concerning the effects of metals on aquatic invertebrates is rather limited. This lack may be due to studies that have shown these forms to be relatively insensitive to various metals (Warnick & Bell, 1969; Rehwoldt *et al.*, 1973; Nehring, 1976). Although most of this work has been conducted on a

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short-term basis (96 h), studies by Arthur & Leonard (1970) and Biesinger & Christensen (1972) show that the sensitivity of some invertebrates to metals is similar to or greater than that of fish when exposed for periods longer than 96 h.

The objective of this study was to re-examine with 28-day exposure periods the effects of cadmium and lead on the survival of some aquatic invertebrates and to determine the bioaccumulation of these metals. Cadmium and lead were selected because data are available concerning their toxicity to aquatic life and because both metals are toxic to fish at low concentrations (Benoit *et al.*, 1976; Davies *et al.*, 1976; Holcombe *et al.*, 1976; Spehar, 1976).

MATERIALS AND METHODS

Water characteristics

Untreated Lake Superior water was used in both cadmium and lead tests at 15 °C. Chemical characteristics of the test water were determined weekly according to methods described by the American Public Health Association *et al.* (1975). Ranges for these measurements were (in mg/litre): dissolved oxygen, 10·1 to 11·0; hardness, 44 to 48 as $CaCO_3$; alkalinity, 40 to 44 as $CaCO_3$ and acidity 1·9 to 3·0 as $CaCO_3$. The pH ranged from 7·1 to 7·7. Turbidity of the test water was measured in nephelometric turbidity units (NTU) (Pijanowski, 1976) on a HACH model 2100A turbidimeter. Normal turbidity for Lake Superior water is approximately 1·0 NTU, but high turbidity (up to 46 NTU) of the test water occurred during approximately two weeks of the lead exposure.

Exposure system

Both tests were conducted with intermittent-flow exposure systems. For the cadmium test a multi-channel toxicant injection system (DeFoe, 1975) was used to deliver five toxicant concentrations and a control to duplicate exposure chambers. The exposure system for the lead test consisted of a proportional diluter (Mount & Brungs, 1967). Toxicant concentrations for both metals were selected in the range used for fish-toxicity testing. The test chambers were glass aquaria, 30 × 60 × 30 cm, with a water volume of 43 litres. Flow rate to each chamber was 500 ml every 2 min providing 95 % replacement of the test water every 9 h (Sprague, 1969).

Fluorescent bulbs provided a light intensity of 10 to 15 lumens at the water surface. Lights were automatically controlled, and the daily photoperiod was set for seasonal light conditions at Duluth, Minnesota.

Toxicant solution

Stock solutions were prepared by dissolving reagent grade $CdCl_2$ and $Pb(NO_3)_2$ in deionised water. Samples for water analysis were taken once a week from the

middle of each glass aquarium and acidified with 0·1 % nitric acid. Samples were analysed by atomic absorption spectrophotometry. Measured cadmium and lead concentrations and standard deviations are included in Figs. 1 and 5, respectively. To verify the accuracy of the method of analysis, known amounts of each metal were added to control water to obtain percentage recoveries. Mean percentage recovery and standard deviation were 91 ± 0·8 for 16 cadmium samples and 100 ± 6·6 for six lead samples. Additionally, one set of samples for each metal was filtered through a 0·1-μm filter to characterise the portion of dissolved metal. Mean percentages of dissolved cadmium and lead ranged from 82 to 90.

Biological procedures

The cadmium test was conducted in January 1975 and the lead test in November 1975. Each test lasted for 28 days. Species exposed to cadmium were: stonefly (*Pteronarcys dorsata*), caddisfly (*Hydropsyche betteni*), mayfly (*Ephemerella* sp.) and snail (*Physa integra*). Those exposed to lead were: stonefly (*P. dorsata*), caddisfly (*Brachycentrus* sp.), snail (*P. integra*) and amphipod (*Gammarus pseudolimnaeus*). Measured lengths of test animals were (in millimetres): stoneflies, 20 to 40; mayflies, 5 to 8; caddisflies, 5 to 8; snails, 6 to 15 and amphipods, 5 to 7. All test animals were collected from the North Shore streams of Lake Superior near Duluth, Minnesota, or from the Eau Clair River near Gordon, Wisconsin. Acclimatisation time in the laboratory holding tanks varied from 1 to 3 weeks. Water temperatures during acclimation ranged from approximately 10 °C to the test temperature of 15 °C.

Ten animals of each species were used in each duplicate chamber. All animals were placed in round stainless steel screen containers (6·5 cm diameter × 15 cm deep) that were suspended in the test chambers. Food for most test animals consisted of autumn-fallen birch and aspen leaves presoaked in diluent water. *Brachycentrus* sp. was fed thawed adult brine shrimp, and *H. betteni* was fed a suspension of *Daphnia* food described by Biesinger & Christensen (1972). All test species were observed once a day for the first 4 days and then once a week for the remainder of the test. In these tests death was defined as complete immobilisation and failure of the animals to respond to probing.

Residue analysis

At the end of the test all animals were rinsed in control water and frozen for residue analysis. Five individuals were analysed per concentration in each test except for caddisflies, which were pooled in groups of five because of their small size (less than 0·02 g, dry weight). Whole animals were analysed by using the wet digestion method described by Leonard (1971). After digestion, residues were assayed by flame atomic absorption spectrophotometry. The accuracy of the method was checked by adding known amounts of each metal to the digestion procedure. Recovery of both metals averaged 95 to 100 %.

Statistical analysis

Mortality data were analysed by using either the manual procedure of Litchfield & Wilcoxon (1949) or a computerised program of Finney (1971) modified by R. W. Andrew, Environmental Research Laboratory-Duluth, Minnesota, USA and K. A. Busch, National Institute of Occupational Safety and Health, Cincinnati, Ohio, USA. In addition, survival and metal-accumulation data were subjected to one-way analysis of variance and Dunnett's two-tailed comparison of treatment means to control means (Steel & Torrie, 1960).

RESULTS

Cadmium

Toxicity. Survival of snails decreased significantly at 85·5 μg/litre after 7 days of exposure (Fig. 1). After 21 days of exposure survival was significantly decreased ($p = 0.05$) at 27·5 μg/litre, the next lower concentration. Some snails exposed to these concentrations developed a condition in which the snail was extended from the shell but was unable to attach the foot or crawl. A white mucus layer covered the exposed foot region of some snails that died.

The toxicity curve relating median lethal concentration (LC50) of cadmium to exposure time for snails is illustrated in Fig. 2. Although concentrations were not high enough to obtain a 4-day LC50 value, the 7-day LC50 of 114 μg/litre was approximately 11 times higher than the 28-day LC50 value of 10·4 μg/litre.

Although mayfly mortality was seen throughout the exposure period, significant effects ($p = 0.05$) on survival were not observed until the last week of exposure (Fig. 3). By this period 70% mortality occurred in the lowest concentration (3 μg/litre), and increased mortality was observed in all higher concentrations. Since mortality was significant only during the last week of exposure, the toxicity curve relating LC50 to exposure time could not be drawn for the mayfly response to cadmium. Since mortality was greater than 50% in the lowest concentration, the estimated LC50 for mayflies after 28 days of exposure was < 3.0 μg Cd/litre.

Cadmium concentrations in this study were not high enough to cause significant decreases in survival of stoneflies or caddisflies after 28 days of exposure. The behaviour of caddisflies at 85·5 and 238 μg Cd/litre, however, was different from that of the controls. The affected larvae showed a vigorous curling motion and were usually free-swimming. Essentially all control larvae were observed in larval cases and responded slowly to probing.

Accumulation. Stoneflies, caddisflies and snails exposed to cadmium concentrations of 3 μg/litre and above for 28 days accumulated significant amounts of cadmium (Fig. 4). No residue values are shown for mayflies because of high mortality in the last week of exposure.

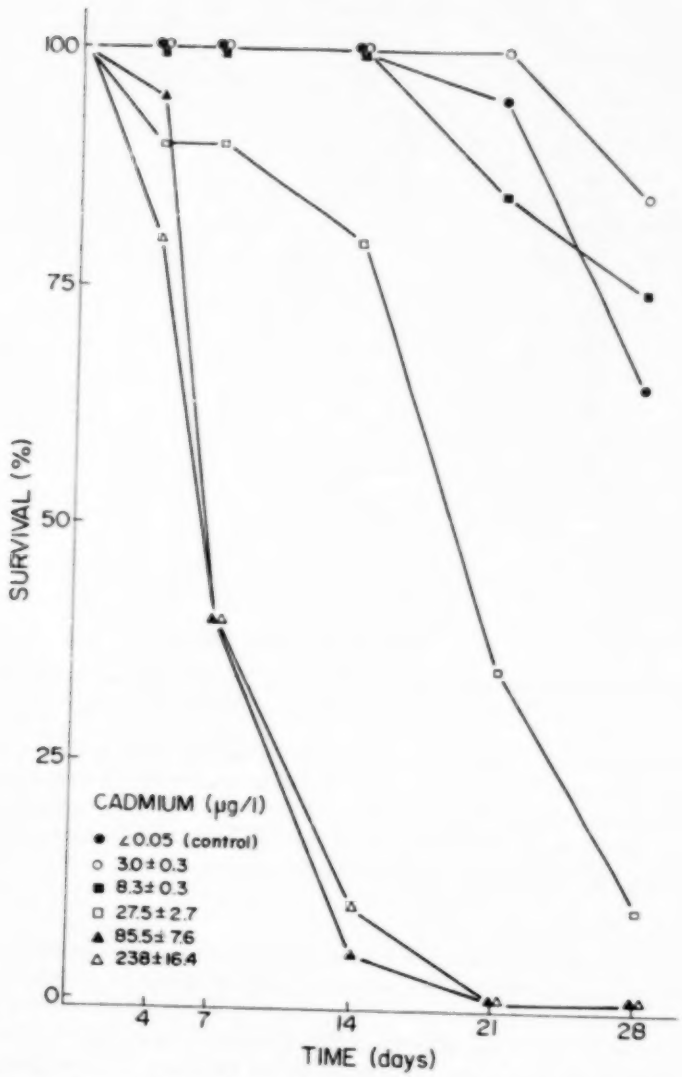

Fig. 1. Percent survival of *Physa integra* after 28 days of exposure to cadmium.

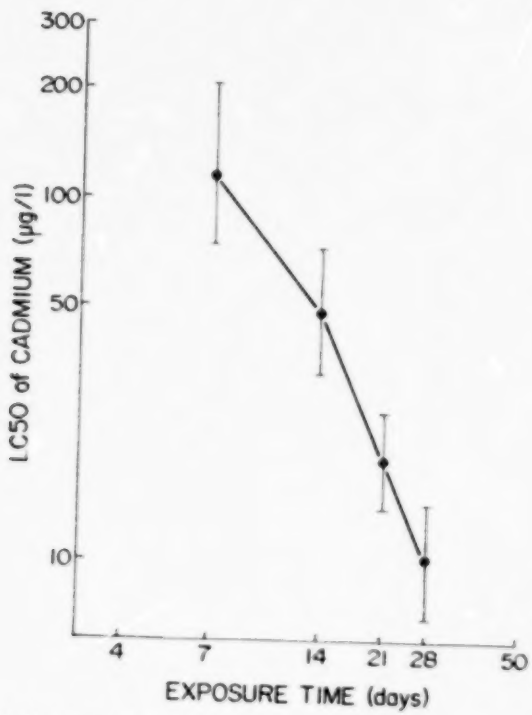

Fig. 2. Relationship between median lethal concentration (LC50) of cadmium and exposure time for *Physa integra*. Bars indicate 95% confidence limits.

Cadmium residues in the animals were 600 to 30,000 times greater than respective cadmium concentrations in the water. Residues in stoneflies and snails increased with increased cadmium concentrations in the water. Cadmium residues in the caddisfly *H. betteni*, however, appeared to reach an equilibrium (on a microgram/gram basis) with higher exposure concentrations.

Lead

Toxicity. Lead was acutely toxic to amphipods and caused greater than 50% mortality at concentrations of 136 µg/litre and above after 4 days of exposure (Fig. 5). By the end of the 28-day test mortality was 60% in the lowest concentration (32 µg/litre), and nearly all animals had died in all higher concentrations. The toxicity curve relating median lethal concentrations to exposure time for lead-exposed amphipods is given in Fig. 6. The 28-day LC50 of 28·4 µg/litre for this species was approximately 4 times lower than the calculated 4-day LC50 value of 124 µg/litre.

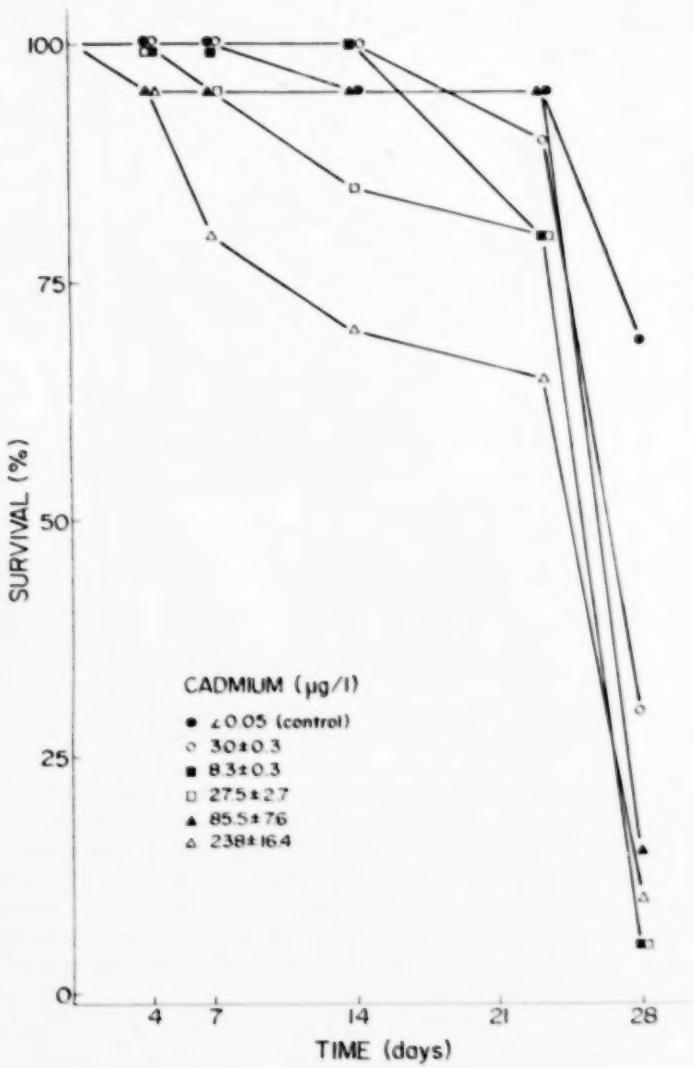

Fig. 3. Percent survival of *Ephemerella* sp. after 28 days of exposure to cadmium.

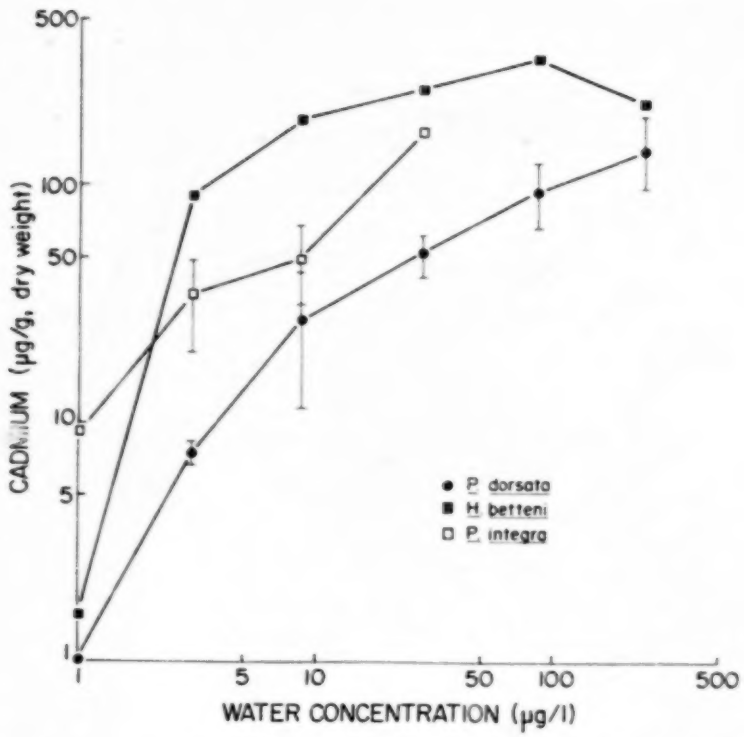

Fig. 4. Cadmium residues (log scale) measured from aquatic invertebrates exposed to cadmium for 28 days. Symbol and bars indicate mean ± SD. Symbols with no bars indicate mean of pooled samples.

Lead concentrations as high as 565 µg/litre did not cause significant decreases in survival of stoneflies, caddisflies and snails after 28 days of exposure.

Accumulation. Invertebrates exposed for 28 days to lead concentrations of 32 µg/litre and above accumulated significant amounts of lead (Fig. 7). Average lead residues in all species were approximately 1000 to 9000 times greater than corresponding water concentrations after 28 days. Residues increased with increased lead concentrations in the water but were not significantly different between species after the 28-day test period.

DISCUSSION

Results of this study suggest that some aquatic invertebrates are affected at much

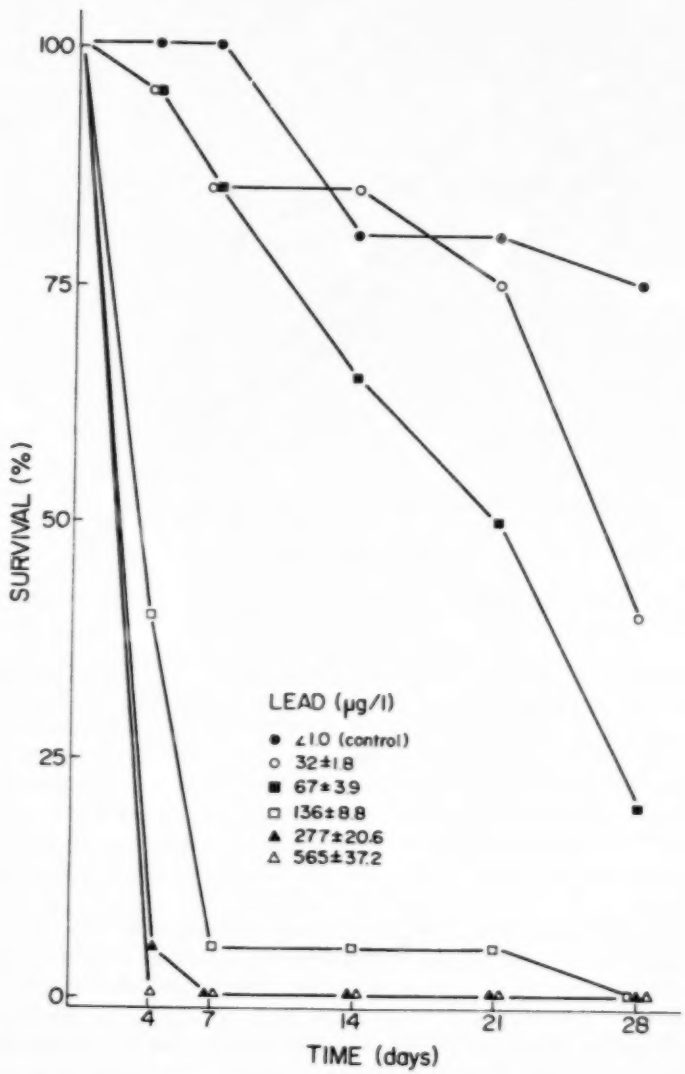

Fig. 5. Percent survival of *G. pseudolimnaeus* after 28 days of exposure to lead.

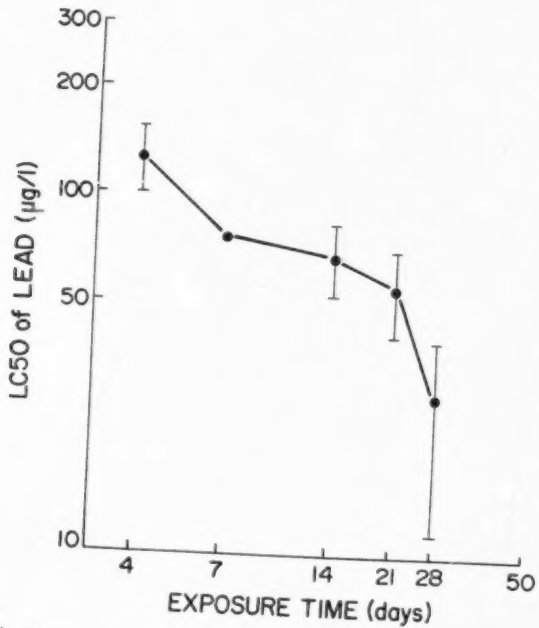

Fig. 6. Relationship between median lethal concentration (LC50) of lead and exposure time for *G. pseudolimnaeus*. Bars indicate 95% confidence limits.

lower concentrations of cadmium and lead when exposed for 28 days than after 4-day (96-h) exposure periods (Figs. 2 and 6). Twenty-eight-day LC50 values for cadmium-exposed snails and lead-exposed amphipods were approximately 11 and 4 times lower than the 7- and 4-day values for these metals, respectively. Median lethal concentrations reported by Warnick & Bell (1969) for mayflies (*Ephemerella* sp.) and caddisflies (*Hydropsyche betteni*) exposed to cadmium for 4 and 10 days, respectively, in Lake Superior water were approximately 400 to 600 times greater than cadmium concentrations, causing adverse effects to the same species in this test after 28 days.

Since lethal thresholds were not observed for species exposed to cadmium or lead after 28 days, it is possible that the median lethal concentrations for these species would decrease if they were exposed for longer periods. Possible adverse effects could occur at concentrations equal to or below those causing adverse effects in fish. For comparison, the lowest effect concentrations obtained for invertebrate species in these tests and those for fish reported by other investigators are shown in Table 1. The lowest effect concentrations of cadmium and lead to invertebrates after 28 days were similar to those of fish exposed to these metals over their complete life cycle in water, of similar water quality.

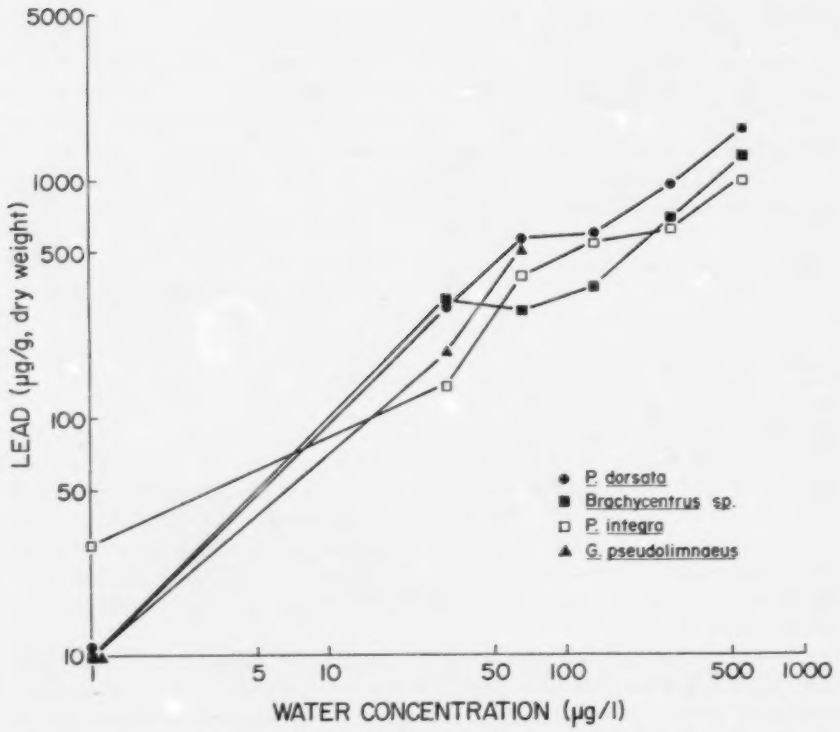

Fig. 7. Lead residues (log scale) measured from aquatic invertebrates exposed to lead for 28 days. Symbols indicate the mean of 5 samples.

Cadmium exposures with stoneflies, and lead exposures with stoneflies, caddisflies and snails suggest that some aquatic invertebrates are relatively insensitive to these metals even after 28-day exposures. The insensitivity of lead-exposed species, however, may be attributed to the high turbidity of the incoming water during this test. Although the effects of suspended solids on metal toxicity have not been studied extensively, Brown *et al.* (1974) observed a direct relationship between concentrations of suspended organic solids and reduction in copper toxicity to rainbow trout (*Salmo gairdneri*).

The sensitivity of invertebrates to cadmium and lead may have been greater if other phases of their life cycle had been studied. No attempt was made to study the most sensitive life stages of the species in this test. Wier & Walter (1976) found that immature snails (*Physa gyrina*) were three times more sensitive to cadmium than mature snails. Clubb *et al.* (1975) similarly observed that insects in the early stages of development were more sensitive to cadmium than at later stages and that moulting

TABLE 1
LOWEST EFFECT CONCENTRATIONS FOR SEVERAL AQUATIC SPECIES EXPOSED TO CADMIUM AND LEAD

Metal	*Lowest effect concentration (μg/l)*	*Exposure period*	*Water hardness (mg/l as $CaCO_3$)*	*Species*
Cadmium ($CdCl_2$)	3·0	28 days	45	*Ephemerella* sp.[a]
	27·5	28 days	45	*Physa integra*[a]
	8·1	100 days	45	*Jordanella floridae*[b]
	3·4	3 years	45	*Salvelinus fontinalis*[c]
Lead ($Pb(NO_3)_2$)	32	28 days	45	*Gammarus pseudolimnaeus*[a]
	119	3 years	44	*Salvelinus fontinalis*[d]
	7·6	2–3 years	28	*Salmo gairdneri*[e]

[a] Values from this test.
[b] Spehar (1976).
[c] Benoit *et al.* (1976).
[d] Holcombe *et al.* (1976).
[e] Davies *et al.* (1976).

was reduced in insects subjected to cadmium. Studies by Pickering & Gast (1972), Eaton (1974) and Benoit *et al.* (1976) have shown that early life stages (embryos and larvae) of fish are the most sensitive to cadmium. Additional studies involving longer toxicant exposures than the commonly used 96-h tests and including other life stages are needed to assess more accurately the impact of heavy metal on aquatic invertebrates.

Abnormal behaviour of snails exposed to cadmium usually preceded death. The condition in which the snail is extended from the shell but is unable to attach the foot or crawl is termed distress (Harry & Aldrich, 1963). These authors observed this condition in the planorbid snail (*Taphius globratus*) exposed to 50, 100, and 100 μg/litre of cadmium, copper and zinc, respectively. Yager & Harry (1964) also observed this condition in the same snail exposed to radioactive isotopes of cadmium, copper and zinc. They concluded that metal ions were effective in producing distress by disrupting membrane permeability. The formation of mucus around the shell aperture of dead or dying snails shown in this test was similarly observed by Sullivan & Cheng (1975) for snails (*Biomphalaria glabrata*) exposed to copper.

Abnormal behaviour in the form of a vigorous curling motion was observed for caddisflies exposed to 85·5 and 238 μg/litre of cadmium. This response was similar to the convulsive behaviour observed in several species of cadmium-exposed fish (Cearley & Coleman, 1974; Eaton, 1974; Benoit *et al.*, 1976; Spehar, 1976).

Relationships between cadmium and lead tissue residues and water concentration were similar in this test to those described for other invertebrate species (Clubb *et al.*, 1975; Nehring, 1976). Metal concentrations in the animals increased with increasing water concentrations for most test species. Although cadmium

concentrations in caddisflies appeared to reach an equilibrium similar to that observed for cadmium-exposed fish (Benoit *et al.*, 1976; Spehar, 1976), residue concentrations were up to 30,000 times greater than respective cadmium concentrations in the test water. Lead residues were as high as 9000 times greater than respective lead water concentrations.

The residue values in cadmium-exposed invertebrates of this test show that they accumulated up to three times more cadmium than flagfish (Spehar, 1976) exposed to this metal for the same period of time and in the same water. Similar comparisons with lead are not available. However, the high accumulations of metals like cadmium in invertebrates are important since they may contribute a major source of metal contamination in the food chain.

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